NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF STODDARD SOLVENT IIC

(CAS NO. 64742-88-7)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

Scheduled Peer Review Date: May 22, 2003

NOTICE

This is a DRAFT Technical Report prepared for public review and comment. Until this DRAFT has been reviewed and approved by the NTP Board of Scientific Counselors' Technical Reports Review Subcommittee in public session, the interpretations described herein do not represent the official scientific position of the National Toxicology Program. Following peer review, readers should contact the NTP for the final version of this Technical Report.

NTP TR 519

NIH Publication No. 03-4453

National Toxicology Program

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Details about ongoing and completed NTP studies are available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov. Abstracts of all NTP Technical Reports and full versions of the most recent reports and other publications are available from the NIEHS' Environmental Health Perspectives (EHP) http://ehp.niehs.nih.gov (866-541-3841 or 919-653-2590). In addition, printed copies of these reports are available from EHP as supplies last.

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CONTENTS

ABSTRACT

Stoddard Solvent IIC

CAS No. 64742-88-7

Synonyms: Medium aliphatic solvent naphtha (petroleum); white spirit

Stoddard solvent (white spirit/mineral spirit) is the most widely used solvent in the paint industry. It is used as a dry cleaning agent and as an extraction, cleaning, and degreasing solvent, as a solvent in aerosols, paints, wood preservatives, asphalt products, lacquers, and varnishes. Stoddard solvent IIC was nominated by the International Union, United Auto Workers, for carcinogenicity testing because of the large volume used in industrial and other settings. Male and female F344/N rats and B6C3F$_1$ mice were exposed to Stoddard solvent IIC (greater than 99% pure) by inhalation for 2 weeks, 3 months, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and mouse peripheral blood erythrocytes.

2-Week Study in Rats

Groups of five male and five female rats were exposed to Stoddard solvent IIC by inhalation at concentrations of 0, 138, 275, 550, 1,100, or 2,200 mg/m^3, 6 hours per day, 5 days per week for 16 days. All rats survived to the end of the study, and mean body weights of all exposed groups were similar to those of the chamber controls. Liver weights of males exposed to 550 mg/m^3 or greater and of females exposed to 275 mg/m^3 or greater were increased. Minimal diffuse cytoplasmic vacuolization of hepatocytes of the liver occurred in all females exposed to 2,200 mg/m^3.

2-Week Study in Mice

Groups of five male and five female mice were exposed to Stoddard solvent IIC by inhalation at concentrations of 0, 138, 275, 550, 1,100, or 2,200 mg/m^3, 6 hours per day, 5 days per week for 17 days. All mice survived to the end of the study, and mean body weights of all exposed groups were similar to those of the chamber controls. Liver weights of males and females exposed to 275 mg/m^3 or greater were significantly increased. Cytomegaly of the liver occurred in all males and females exposed to 2,200 mg/m^3.

3-Month Study in Rats

Groups of 10 male and 10 female rats were exposed to Stoddard solvent IIC by inhalation at concentrations of 0, 138, 275, 550, 1,100, or 2,200 mg/m^3, 6 hours per day, 5 days per week for 14 weeks. All rats survived to the end of the study, and the final mean body weight of females exposed to 275 mg/m^3 was greater than that of the chamber controls. The relative kidney, liver, and testis weights of all exposed groups of males and the absolute kidney weights of males exposed to 550 mg/m^3 or greater were increased. The incidences of renal tubule granular casts were significantly increased in males exposed to 550 mg/m^3 or greater, and the severities of renal tubule hyaline droplet accumulation, granular casts, and regeneration increased with increasing exposure concentration in males. The incidences of goblet cell hypertrophy of the nasal respiratory epithelium in males and females exposed to 2,200 mg/m^3 were significantly increased. Sperm motility was decreased in males exposed to 550 mg/m^3 or greater.

3-Month Study in Mice

Groups of 10 male and 10 female mice were exposed to Stoddard solvent IIC by inhalation at concentrations of 0, 138, 275, 550, 1,100, or 2,200 mg/m^3, 6 hours per day, 5 days per week for 14 weeks. Mean body weights of exposed groups were similar to those of the chamber controls, but liver weights of males exposed to 2,200 mg/m^3 were significantly increased, and their sperm motility was significantly decreased. The incidences of hematopoietic cell proliferation of the spleen in all exposed groups of females were greater than that in the chamber controls.

2-Year Study in Rats

Groups of 50 male and 50 female rats were exposed to Stoddard solvent IIC by inhalation at concentrations of 0, 138 (males), 550, 1,100, or 2,200 (females) mg/m^3, 6 hours per day, 5 days per week for 105 weeks. Additional groups of 10 males and 10 females were exposed to the same concentrations for 3 months for renal toxicity analyses. Survival in the top exposure concentration groups of males and females was significantly less than that of the chamber controls. Mean body weights of exposed males and females were similar to those of the chamber controls.

Cell proliferation analyses were performed in the left kidney of males and females after 3 months of exposure. The mean numbers of labeled cells and the labeling indices in males exposed to 550 and 1,100 mg/m^3 were significantly increased. The amount of α2u-globulin in the right kidney of males increased with increasing exposure concentration. Also, the incidences of granular casts and cortical tubule degeneration and regeneration were generally increased in exposed males, as was the severity of hyaline droplets. These effects did not occur in females.

At 2 years, the incidences of benign and benign or malignant pheochromocytoma (combined) of the adrenal medulla occurred with positive trends in males, and the incidences in the 550 and 1,100 mg/m^3 groups were significantly increased. Due to increased incidences of renal tubule hyperplasia in males at 2 years, extended kidney evaluations were conducted; a slightly increased incidence of renal tubule adenoma occurred in the 1,100 mg/m^3 group. Nonneoplastic lesions related to Stoddard solvent IIC exposure occurred in the kidney of males.

2-Year Study in Mice

Groups of 50 male and 50 female mice were exposed to Stoddard solvent IIC by inhalation at concentrations of 0, 550, 1,100, or 2,200 mg/m^3, 6 hours per day, 5 days per week for 105 weeks. Survival of exposed mice was similar to that of the chamber controls. Mean body weights of exposed females were greater than those of the

chamber controls. The incidences of hepatocellular adenoma occurred with a positive trend in females, and the incidence of multiple hepatocellular adenoma in females exposed to 2,200 mg/m^3 was significantly increased. However, the incidences of hepatocellular adenoma or carcinoma (combined) and hepatocellular carcinoma alone in exposed males and females were not significantly increased.

Genetic Toxicology

Stoddard solvent IIC was tested for mutagenicity in *Salmonella typhimurium* strains TA97, TA98, TA100, and TA1535, with and without S9 metabolic activation enzymes; all results were negative. *In vivo*, the frequency of micronucleated erythrocytes was assessed in peripheral blood samples from male and female B6C3F$_1$ mice after 3 months of inhalation exposure to Stoddard solvent IIC, and results were negative.

Conclusions

Under the conditions of this 2-year inhalation study, there was *some evidence of carcinogenic activity** of Stoddard solvent IIC in male F344/N rats based on increased incidences of adrenal medulla neoplasms; the slightly increased incidences of renal tubule adenoma may have been related to Stoddard solvent IIC exposure. There was *no evidence of carcinogenic activity* of Stoddard solvent IIC in female F344/N rats exposed to 550, 1,100, or 2,200 mg/m^3. There was *no evidence of carcinogenic activity* of Stoddard solvent IIC in male B6C3F$_1$ mice exposed to 550, 1,100, or 2,200 mg/m^3. There was *equivocal evidence of carcinogenic activity* of Stoddard solvent IIC in female B6C3F$_1$ mice based on increased incidences of hepatocellular adenoma; this slight increase was associated with increased body weight in exposed females.

Exposure of male rats to Stoddard solvent IIC resulted in nonneoplastic lesions of the kidney characteristic of α2u-globulin accumulation.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Stoddard Solvent IIC

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Concentrations in air	Chamber control, 138, 550, or 1,100 mg/m^3	Chamber control, 550, 1,100, or 2,200 mg/m^3	Chamber control, 550, 1,100, or 2,200 mg/m^3	Chamber control, 550, 1,100, or 2,200 mg/m^3
Survival rates	29/50, 19/50, 21/50, 16/50	36/50, 30/50, 32/50, 25/50	34/50, 32/50, 27/50, 32/50	36/50, 34/50, 27/50, 34/50
Body weights	Exposed groups similar to the chamber control group	Exposed groups similar to the chamber control group	Exposed groups similar to the chamber control group	Exposed groups greater than the chamber control group
Nonneoplastic effects	Kidney: renal tubule hyperplasia (0/50, 1/50, 8/50, 23/50); transitional epithelial hyperplasia (0/50, 2/50, 8/50, 5/50); papilla mineralization (1/50, 8/50, 30/50, 39/50); severity of chronic nephropathy (2.0, 2.3, 2.5, 2.8)	None	None	None
Neoplastic effects	Adrenal medulla: benign pheochromocytoma (5/50, 9/50, 13/50, 17/50); benign or malignant pheochromocytoma (6/50, 9/50, 13/50, 19/50)	None	None	None
Equivocal findings	Kidney: (standard and extended evaluation combined) renal tubule adenoma (3/50, 2/50, 3/50, 7/50)	None	None	Liver: hepatocellular adenoma (9/50, 12/50, 15/50, 18/50)
Level of evidence of carcinogenic activity	Some evidence	No evidence	No evidence	Equivocal evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:	Negative in strains TA97, TA98, TA100, and TA1535, with and without S9			
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo:*	Negative in males and females			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence);** one category for uncertain findings **(equivocal evidence);** one category for no observable effects **(no evidence);** and one category for experiments that cannot be evaluated because of major flaws **(inadequate study).** These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as "were also related" to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as "may have been" related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on Stoddard solvent IIC on May 22, 2003, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

NOTE: A summary of the Technical Reports Review Subcommittee's remarks will appear in a future draft of this report.